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ROBERT E. HANSON
FULBRIGHT & JAWORSKI L.L.P
600 CONGRESS AVENUE
SUITE 2400
AUSTIN, TX 78701

EXAMINER	
KALLIS, RUSSELL	
ART UNIT	PAPER NUMBER
1638	13

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/885,723	KARUNANANDAA ET AL.	
	Examiner	Art Unit	
	Russell Kallis	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 January 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-77* is/are pending in the application.

4a) Of the above claim(s) *34-37,39,41,43-62 and 69* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-35,38,40,42 and 63-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4,5* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group I, Claims 1-35, 38, 40, 42 and 63-68 in Paper No. 12 is acknowledged. The traversal is on the ground(s) that the claim groups I-VII and XII-XVII comprise the same claims. This is not found persuasive because the said claim groups are drawn to inventions that have different combinations of enzymes that would require different searches and present an undue burden upon the Examiner.

The requirement is still deemed proper and is therefore made FINAL.

Claim Objections

Claims 1, 6, 12, 18, 20, 25-29 and dependents are objected to because they encompass non-elected subject matter.

Claims 40 and 42 are objected to because of the following informalities: The claims are dependent upon the non-elected subject matter of Claim 37. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-35, 38, 40, 42, and 63-68 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant broadly claims a DNA sequence encoding a polypeptide having HMG-CoA activity, a DNA sequence encoding a polypeptide having squalene epoxidase activity, and a DNA sequence encoding a tocopherol synthesis pathway enzyme.

Applicant describes HMG-CoA reductases on page 59 of the specification; *Arabidopsis* and *Brassica* squalene epoxidase cDNA on page 72, and full length cDNA of SEQ ID NO: 4 and 6 encoding a putative squalene epoxidase from *Arabidopsis* on page 73 of the specification and in Example 5 on page 142.

Applicant does not describe the broad categories of a DNA sequence encoding a polypeptide having HMG-CoA activity, a DNA sequence encoding a polypeptide having squalene epoxidase activity, and a DNA sequence encoding any tocopherol synthesis pathway enzyme.

Given the claim breadth and lack of guidance as discussed above, the specification does not provide an adequate written description of the claimed invention.

See *University of California V. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism which would encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism.

The court also addressed the manner by which genus of cDNAs might be described: "A description of a genus of cDNAs may be achieved by means of a recitation of a representative

number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." *Id.* At 1406.

Given the failure of the recombinant DNA encoding HMG-CoA reductase activity, squalene epoxidase activity, a SAM gamma tocopherol methyltransferase or any tocopherol synthesis pathway enzyme to be adequately described, methods of its use are also inadequately described. See Written Description Guidelines, Federal Register Vol. 66 No. 4, Friday January 5, 2001 "Notices", pages 1099-1111.

Claims 1-35, 38, 40, 42, and 63-68 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Applicant broadly claims recombinant constructs comprising hetrologous DNA encoding HMG-CoA reductase activity and squalene epoxidase activity or said recombinant construct further comprising a DNA encoding SAM methyltransferase activity or any "tocopherol synthesis pathway enzyme" under control of seed specific promoters; transformed host cells, and transformed plants comprising said recombinant constructs, and storage organs comprising a transformed host cell, plants having either elevated or decreased levels of steroid/sterol pathway products using said recombinant constructs and a process of increasing or decreasing steroid/sterol formation in transformed host cells and plants.

Applicant teaches enhancement of phytosterol content in soybean seed by specific expression in soybean seeds of full length HMG-CoA reductase cDNA from rubber (Example 1

page 129); enhancement of phytosterol content in soybean seed by specific expression in soybean seeds of a partial cDNA encoding the catalytic region of HMG-CoA reductase from rubber (Example 2 page 131); a comparison of phytosterol accumulation in the seeds of soybean transformed with a partial cDNA encoding the catalytic region of HMG-CoA reductase from *Arabidoipsis*, and soybean transformed with a partial cDNA encoding the catalytic region of HMG-CoA reductase from *Arabidoipsis* and the SMT II cDNA from *Arabidopsis* that showed significant accumulation in both instances (Example 2 pages 132-139); enhancement of phytosterol in the seeds of *Arabidopsis* plants transformed with various forms of *Arabidopsis* and rubber HMG-CoA reductase encoding DNA (Example 3 pages 140-141); and the effect on sterol content in yeast HMGR1 knockout mutants expressing various HMGR constructs of *Arabidopsis* and rubber (Example 4 pages 141-142).

Applicant does not teach a plant transformed with a recombinant hetrologous plant transformation construct comprising any DNA encoding any HMG-CoA reductase activity from any plant and squalene epoxidase activity or a plant transformed with a recombinant hetrologous plant transformation construct comprising DNA encoding HMG-CoA reductase activity, squalene epoxidase activity, and SAM methyltransferase activity from any plant, or any other gene encoding any "tocopherol synthesis pathway enzyme".

The isolation of orthologous HMG-CoA reductase, suqalene epoxidase, or SAM methyltransferase encoding DNA sequences from other species introduces an element of unpredictability. The limitation is introduced in finding homologous regions that would adequately enable either PCR amplification or southern hybridization and would entail using either degenerate primers or probes with limited sequence identity. Thus the screen for

orthologous sequences would isolate many genes other than those of interest. The inherent unpredictability in isolation of a homologous sequence encoding the same protein activity is illustrated in an example where a small number of changes to the coding region for a strict desaturase resulted in an enzyme with a hydroxylase activity and that a small number of changes to the coding region of a desaturase could account for the functional divergence seen across a range of enzymes involved in fatty acid metabolism (Broun P. *et al.* Science Vol. 282; 13 November 1998, pp. 1315-1317; Abstract lines 4-6 and p. 1317 column 1, lines 37-56).

Further, isolating DNA fragments using stringent hybridization conditions, does not always select for DNA fragments whose contiguous nucleotide sequence is the same or nearly the same as the probe. Fourgoux-Nicol et al (1999, Plant Molecular Biology 40 :857-872) teach the isolation of a 674bp fragment using a 497bp probe incorporating stringent hybridization conditions comprising three consecutive 30 minute rinses in 2X, 1X and 0.1X SSC with 0.1% SDS at 65^0C (page 859, left column, 2^{nd} paragraph). Fourgoux-Nicol et al also teach that the probe and isolated DNA fragment exhibited a number of sequence differences comprising a 99bp insertion within the probe and a single nucleotide gap, while the DNA fragment contained 2 single nucleotide gaps and together the fragments contained 27 nucleotide mismatches. Taking into account the insertions, gaps and mismatches, the longest stretch of contiguous nucleotides to which the probe could hybridize consisted of 93bp of DNA (page 862, Figure 2).

Moreover, attempts to engineer biosynthetic flux through a pathway by overexpressing several catalytic steps at once would be blocked by other rate limiting enzymatic reactions upstream of the modified pathway steps. Squalene synthase activity is a potential regulatory point in sterol biosynthesis and is active in the pathway prior to the enzymatic conversions

catalyzed by squalene epoxidase and SAM methyltransferase of the claimed invention. Interestingly, the enzyme is regulated by protein turnover as well as possibly phosphorylation under elicitor conditions that would normally occur in nature suggesting unpredicted limitations in engineering flux through the sterol biosynthetic pathway (Devarenne T. Plant Physiology, July 2002; Vol. 129, pp. 1095-1106; see Abstract and page 1102 column 2, first full paragraph; the entire paragraph).

Given the lack of guidance for isolating any polypeptide having tocopherol pathway activity, orthologous HMG-CoA reductase, squalene epoxidase, or SAM methyltransferase encoding DNA sequences from other species or for producing plants transformed with a recombinant hetrologous plant transformation construct comprising DNA encoding HMG-CoA reductase activity and squalene epoxidase activity or a plant transformed with a recombinant hetrologous plant transformation construct comprising DNA encoding HMG-CoA reductase activity, squalene epoxidase activity, and SAM methyltransferase activity, the breadth of the claims, and given the unpredictability in the art, undue trail and error experimentation would be needed by one skilled in the art to isolate a multitude of non-exemplified cDNA encoding polypeptides having steroid or tocopherol synthesis pathway activity, or to evaluate the ability of a multitude of non-exemplified polypeptides having steroid or tocopherol synthesis pathway activity to alter the phenotype of a multitude of non-exemplified transformed plant species. Therefore, the invention is not enabled.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 20-24 and 26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

At Claim 26, the entire claim, it is not clear how a plant can have an elevated level of total accumulated sterol in lines 1-6 and then have further limitations that would reduce the levels of squalene and all downstream sterol products, considering that squalene is the precursor to all other sterols produced by the pathway. Further, in lines 26-28, "an otherwise identical plant whose genome does not contain introduced DNA encoding said at least one polypeptide having steroid pathway enzyme activity" is a plant that is transformed with HMG-CoA reductase wherein such a plant could have either increased or decreased levels of squalene, or squalene and the other downstream steroid pathway products, depending on whether the gene was expressed or co-suppressed. Moreover, the claim does not set forth the metes and bounds of the claimed invention.

Claim 20 recites the limitation "regenerating said transformed plant cell" in line 31. There is insufficient antecedent basis for this limitation in the claim. Dependent claims are included in the rejection.

Claim 25, lines 17-18, "a non-transformed plant of the same strain" is not art accepted language with respect to plants. The claim should read --a non-transformed plant of the same species--.

Claims 14 and 15 are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should depend upon other claims in the alternative only. Insertion of --one-- after "any" would obviate this rejection. In the interest of compact prosecution, the claims have been treated on their merits. Such treatment does not relieve Applicants of the requirement to respond to this objection. See MPEP § 608.01(n).

Claim Rejections - 35 USC § 101

Claims 15, 17-19, 31-35 and 67-68 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. Claims 15, 17-19 and 67-68 are drawn to either non-transformed plants or storage organs of non-transformed plants, further comprising non-plant "host" cells. Claims 33-34 are drawn to non-transformed plant cells. See *American Wood v. Fiber Distintegrating Co.*, 90 U.S. 566 (1974), *American Fruit Growers v. Brogdex Co.*, 283 U.S. 2 (1931), *Funk Brothers Seed Co. v. Kalo Inoculant Co.*, 33 U.S. 127 (1948), *Diamond v. Chakrabarty*, 206 USPQ 193 (1980). Claims 31-35 are drawn to seeds of a hemizygous transgenic plant that would not necessarily comprise the transgene due to mendelian segregation of the transgene in the first generation of plants after transformation and in subsequent generations that would have only one copy of the transgene. Thus, the claimed inventions encompass untransformed plants and seeds, which are a product of nature and not one of the five classes of patentable subject matter.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-35, 38, 40 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chapell J. *et al.* U.S. Patent 5,589,619 published December 31, 1996 in view of Covello P. *et al.* U.S. Patent 6,153,815 published November 28, 2000, effectively filed January 5, 1999.

Applicant broadly claims recombinant constructs comprising hetrologous DNA encoding HMG-CoA reductase activity and squalene epoxidase activity under control of seed specific promoters; transformed host cells, and transformed plants comprising said recombinant constructs, and storage organs comprising a transformed host cell, plants having either elevated or decreased levels of steroid/sterol pathway products using said recombinant constructs and a process of increasing or decreasing steroid/sterol formation in transformed host cells and plants.

Chappell teaches increased squalene and total sterol accumulation in transgenic tobacco storage organs (i.e. leaves and inherent expression in seeds) expressing a recombinant construct comprising a seed specific Lectin promoter (column 12 lines19-32) and terminator regions and DNA encoding a HMG-CoA reductase (column 25, Table 6) comprising the catalytic region of hamster HMG-CoA reductase of SEQ ID NO: 1 (column 9 lines 29-41).

Chappell does not teach further incorporation of cDNA encoding the downstream sterol biosynthetic enzyme squalene epoxidase.

Covello teaches a *B. napus* cDNA encoding squalene epoxidase in antisense orientation (column 12 lines 28-64) and an increase in squalene accumulation in *Arabidopsis* transformed with *B. napus* cDNA encoding squalene epoxidase in antisense orientation.

It would have been obvious at the time of Applicant's invention to modify the invention of Chappell that teaches increased total sterols in a plant transformed with HMG-CoA reductase to include the *B. napus* cDNA encoding squalene epoxidase in sense or antisense orientation. One of ordinary skill in the art would have been motivated by the knowledge common in the art that transformation vectors comprising cDNA encoding a downstream enzyme of the squalene sterol biosynthetic pathway are valuable materials for genetic engineering of plants to increase the total level of sterols in plants, as made evident by both the success of Christensen and Covello in increasing the total level of sterols and increasing the accumulation of squalene, and that one would have had a reasonable expectation of success of expressing genes in transformed plants and plant cells. Choice of transforming a particular plant species or genotype, including an apomictic genotype, would have been the optimization of process parameters.

All claims are rejected.

Claims 63-68 are deemed free of the prior art, given the failure of the prior art to teach or reasonably suggest a recombinant vector comprising cDNA encoding an HMG-CoA reductase, a squalene epoxidase and a S-adenosylmethionine-dependent gamma tocopherol methyltransferase and plants, transformed with said recombinant construct.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Kallis whose telephone number is (703) 305-5417. The examiner can normally be reached on Monday-Friday 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on (703) 306-3218. The fax phone numbers for the Group is (703) 308-4242 or (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding, or if the examiner cannot be reached as indicated above, should be directed to the receptionist, whose telephone number is (703) 308-0196.

Russell Kallis Ph.D.
March 19, 2003

DAVID T. FOX
PRIMARY EXAMINER
GROUP ~~180~~ 1638